

Characterization of a New Enterococcal Gene, *satG*, Encoding a Putative Acetyltransferase Conferring Resistance to Streptogramin A Compounds

Streptogramin antibiotics are mixtures of two chemically unrelated A and B compounds that act synergistically in vivo against gram-positive pathogens, such as staphylococci, streptococci, and enterococci (8, 11). Resistance against B compounds is very widespread among enterococci and is mediated via the *ermB* gene cluster (e.g., on Tn917) that confers macrolide-lincosamide-streptogramin B resistance (7). The synergistic mixture of streptogramins A and B overcomes resistance to B compounds but is inactive in resistance to A compounds. The only known resistance mechanism against streptogramin A compounds in enterococci is mediated by the streptogramin acetyltransferase SatA (9). *Enterococcus faecium* isolates with *satA*-mediated resistance have been found in samples of human and animal origins, indicating a possible spread of resistance genes or resistant bacteria among different ecosystems (10).

We isolated a quinupristin-dalfopristin-resistant *E. faecium* UW1965 from a sewage treatment plant in Germany. The resistance determinant was transferred to a susceptible recipient, producing the transconjugant UW1965K1. UW1965K1 is resistant to quinupristin-dalfopristin (MIC ≥ 16 μ g/ml) and virginiamycin M (A compound; MIC, 16 μ g/ml), whereas the

MIC of each antibiotic for the recipient was 1 μ g/ml. PCR amplification for the *satA* gene was negative.

In staphylococci, resistance to streptogramin A compounds is mediated by two mechanisms: (i) acetylation of the streptogramin A via acetyltransferases (Vat, VatB, and VatC [1-3]) and (ii) efflux due to an ABC transporter (Vga and VgaB [4, 5]). PCR amplification for the *vat*, *vatB*, *vatC*, and *vga* genes failed to produce any product. The putative protein sequences of the known streptogramin acetyltransferases in staphylococci and enterococci contain three conserved motifs (2). Corresponding primers, *satI* and *satJ*, have been made, producing a 144- to 147-bp fragment for *vat*, *satA*, and *vatB* (2). PCR performed with these primers resulted in a ca. 150-bp fragment for UW1965K1. A digoxigenin-labelled probe of the amplified fragment was prepared, hybridizing with a 5.5-kbp fragment of *EcoRI*-digested plasmid DNA from the transconjugant. The corresponding plasmid fragment was cloned into pUC18 and sequenced.

The resulting DNA sequence (Fig. 1) did not show significant identity with other gene sequences from GenBank on the DNA level (6). One suitable open reading frame (ORF) was found, giving rise to a putative 214-amino-acid (214-aa) pro-

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1  cggtaccogg  ggatccctcta  gactataaatt  aaaattaaat  aactcaattc  ggaggtacta
      start      primor sat01
61  acatgactat  acctgacgca  aatgcaatct  atcctaactc  agccatcaaa  gaggtgtct
      M T I P D A N A I Y P N S A I K E V V F
      aa-motif I
121  ttatcaagaa  cgtgatcaaa  agtcccaata  ttgaattgg  ggactacacc  tattatgatg
      I K N V I K S P N I E Y G D Y T Y Y D D
181  acccagtaaa  tcccaccgat  tttgagaac  acgttaccca  tcactatgaa  tttctaggcg
      P V N P T D F E K H V T H H Y E F L G D
241  acaaattaat  catcggtaaa  ttttgttcta  tcgccagtgg  cattgaattt  atcatgaacg
      K L I I G K F C S I A S G I E F I M N G
      aa-motif II
301  gtgccaaaca  cgtaatgaaa  ggtatttcga  cttatccatt  taatatttta  ggtggcgatt
      A N N H V M K G I S T Y P F N I L G G D W
361  ggcaacaata  cactcctgaa  ctgactgatt  tgccgttgaa  aggtgatact  gtagtggaa
      Q Q Y T P E L T D L P L K G D T V V G N
      aa-motif III
421  atgacgtgtg  gtttgggcaa  aatgtgaccg  tccatccagg  cgtaaaaata  ggtgacggtg
      D V W Y G Q N V T V L P G V K I G D G A
481  ccattatcgg  agcaaatagt  gttgtaacaa  aagacgtcgc  tccatataca  attgtcggtg
      I I G A N S V V T K D V A P Y T I V G G
      primor sat02
541  gcaatccaat  tcaactcatc  ggaccaagat  ttgaaccgga  agttattcaa  gcattagaaa
      N P I Q L I G P R F E P E V I Q A L E N
601  atctggcatg  gtggaataaa  gatattgaat  ggataactgc  taatgttcct  aaactaatgc
      L A W W N K D I E W I T A N V P K L M Q
661  aaacaacacc  cacacttgaa  ttgataaaca  gtttaatgga  aaatgaaaa  caaaaaagcc
      T T P T L E L I N S L M E K
721  gtgcaagcaa  tccaaaaatg  attggttaca  cggcctttac  tatttagtga  atccaattta
781  ttaataatag  atatgatata  ccagtaaaaa  atacactagc  cacctctggc  ggtactctac
841  tcgtatatatt  tatttacgac  cttctgatga  taaaggtcac  ttccctgtcc  ccagaaaaata
901  aagc

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FIG. 1. A 904-bp sequence located on the 5.5-kbp cloned fragment in pUC18 (GenBank accession no. AF139725). The ORF begins at nucleotide 63 with an ATG start codon preceding a putative ribosomal binding site (RBS) (double-underlined) at positions 50 to 57. The predicted gene sequence encodes a protein of 214 aa which shows significant homology with other streptogramin acetyltransferases (aa motifs I, II, III; see also Fig. 2). The locations of the primers *satG1* and *satG2*, specific only for the *satG* sequence, are underlined (plus strand).

Motif I

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SatG: 1  M-----TIPDANAIPNSAIKEVVFQKVI-KSPNIEIGDTTYYDDPVNPTDFEKGVTHHYEFLGDKLI 63
VatB: 1  MK-----YGPDPNSIYPHEEIKSVCFIKNTI-TNPNIIIVGDTTYSVDVNGAEKFEHVTHHYEFLGDKLV 64
VatC: 1  MKWQOQGGPNFEEIYPIEGNKHVQPIKPSI-TKPNILVQETSYTDSK-DGESFESQVLYHYEFLGDKLI 67
SatA: 1  M-----GPNPMQHYPIEGNKSQVQPIKPILEKLENVEVQETSYTDSK-NGETPDQILYHYEFLGDKLI 62
Vat: 1  MNLNNDHGGDPENILPIKGNRNLPQIKPTI-TNENILVQETSYTDSKRGES-FEDQVLYHYEFLGDKLI 67
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Motif II

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SatG: 64  IGKPCSIASGIEFIMNGANERMDGISTYPPNLLGGDWQOYTPEL-TDPLKGDVVGDVNVQGNV 128
VatB: 65  IGKPCAIAGIEFIMNGANERMDGISTYPPNLLGGDWQOYTPEL-TDPLKGDVVGDVNVQGNV 129
VatC: 68  LGKPCSIGPGTTFIMNGANERMDG-STPPFNLFNGWEKATPTL-EDLPYKGTETIGNDVNIQRDV 131
SatA: 63  IGKPCSIGPGVTIIMNGANERMDG-STYPPNLFNGWEKATPTL-DQLPIKGTETIGNDVNIQRDV 126
Vat: 68  IGRPCSIGPGTTFIMNGANERMDG-STYPPHFRNGWEKYNPSL-KDPLKGDIEIGNDVNIQRDV 131
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Motif III

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SatG: 129  TVLPVKIGDGAIGANSVVTKDVAPTYIVGGNPIQLIGRPEPEVQALLENLAW 183
VatB: 130  TVMPGIQIGDGAIVAAANSVVTKDVPYRIIGGNPSRIKKRFEDELIDYLLQIKW 184
VatC: 132  TIMPGVKIGNGAIIAAKSVVTKNDVPYSVGGNPSRLIKIRFSKEIAALLKVRW 186
SatA: 127  VIMPGVKIGDGAIVAAANSVVKDIAPYMLAGGNPANEIKQRFQDQDTINQLLDIKW 181
Vat: 132  TIMPGVKIGDGAIAAEAVVTKNVAPYSIVGGNPLKFIKRFSDGVIEEWLALQW 186
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SatG: 184  WNKDIEWITANVPKLMOTTPTLELINSIMEK 214
VatB: 185  WDMSAOKIFSNETLCSS--DLEKIKSIRD 212
VatC: 187  WDLEIETINENI 198
SatA: 182  WNPIDIINENIDKILDNSIIREVI 206
Vat: 187  WNLDMKIINENLP 199
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FIG. 2. Alignment of amino acid sequences of acetyltransferases from staphylococci and enterococci (1–3, 9) conferring resistance to streptogramin A antibiotics. Identical residues are indicated by asterisks. Highly conserved regions in different streptogramin A acetyltransferases—motifs I, II, and III—are boldfaced. Primers satI and satJ have been designed on the basis of the corresponding nucleotide sequences in motifs II and III (2).

tein. A comparison of amino acid similarities indicated rather significant homology between streptogramin acetyltransferases and the new putative acetyltransferase, designated SatG (Fig. 2). Based on the sequence for *satG*, two primers, satG1 and satG2, have been designed. Preliminary results of a search for streptogramin-resistant enterococci (*E. faecium*, *E. hirae*, and *E. durans*) revealed the existence of the *satG* gene in 9 of 23 isolates from sewage, 6 of 24 isolates from broiler samples, and all 17 isolates from poultry manure. Of 62 quinupristin-dalfopristin-resistant *E. faecium* (QDREF) isolates from hospitals in Germany, 9 were positive for *satG*. The high number of *satG* QDREF isolates from poultry meat and manure may be due to selection of these bacteria by use of virginiamycin as a feed additive, and spread of the resistance via the food chain to humans is very likely. This hypothesis is being investigated.

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